Nanocrystalline Cellulose from Invasive Tunicates

- *Isolation, Characterization and Applications*



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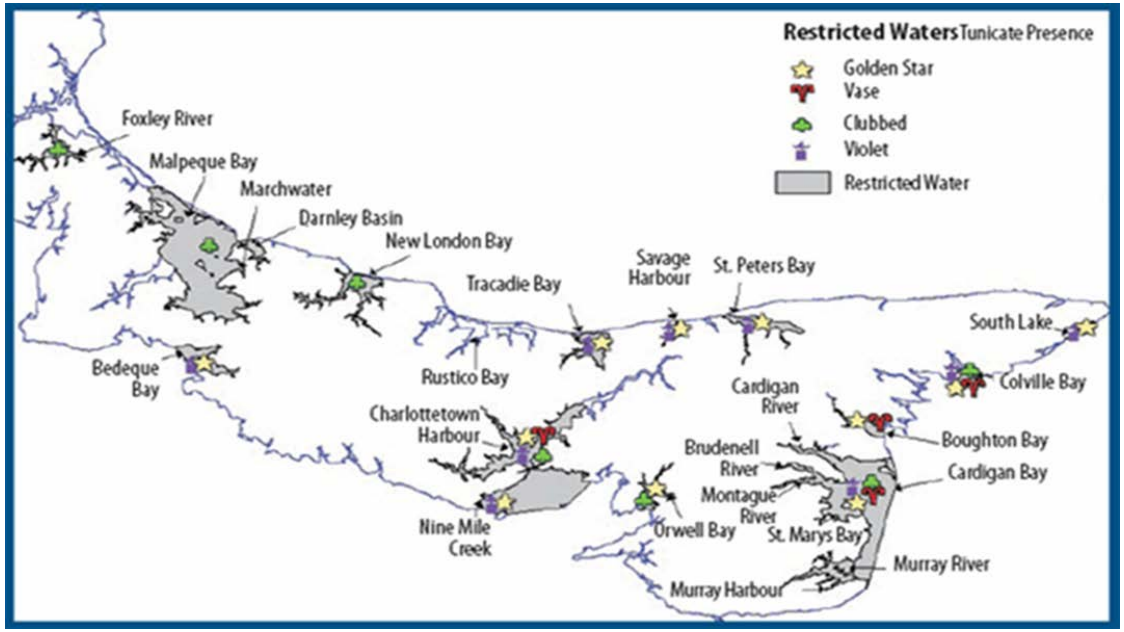
July 24th, 2018

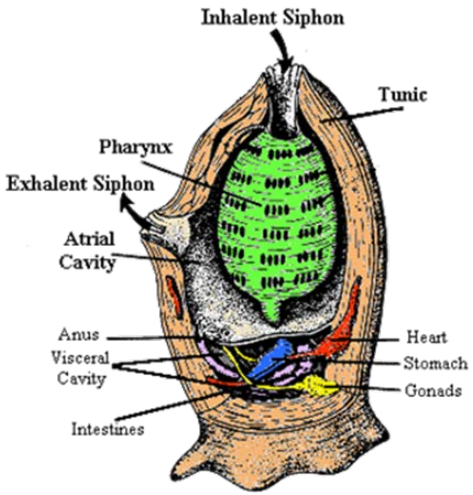
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Invasive Tunicates on PEI







- The first invasive species of tunicate in PEI was documented in 1998.
- There are now 4 invasive species.
- Tunicates can be found in all major island harbours.
- This provides a unique situation on PEI where the supply of tunicates is large.

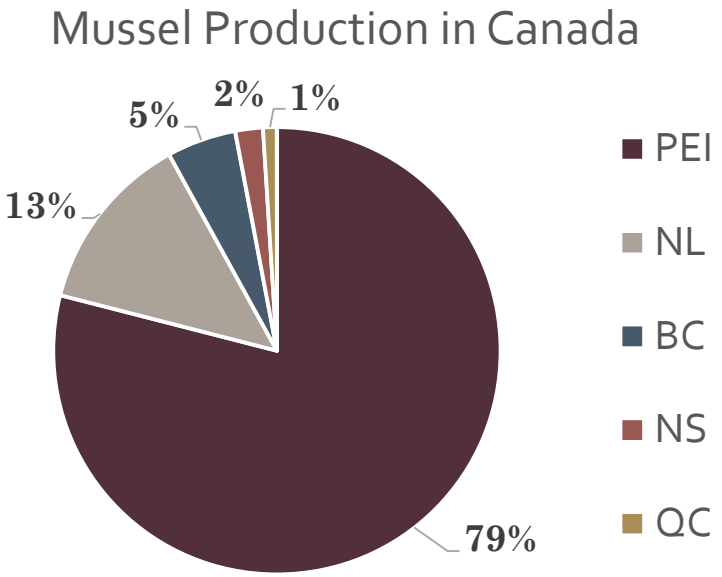
Problems Invasive Tunicates Cause

- Mussel crops in PEI waters are valued at ~$30 million annually.
- Invasive tunicates overgrow mussels and compete for the same food.
- This reduces yields and increases costs of harvesting and processing mussels.



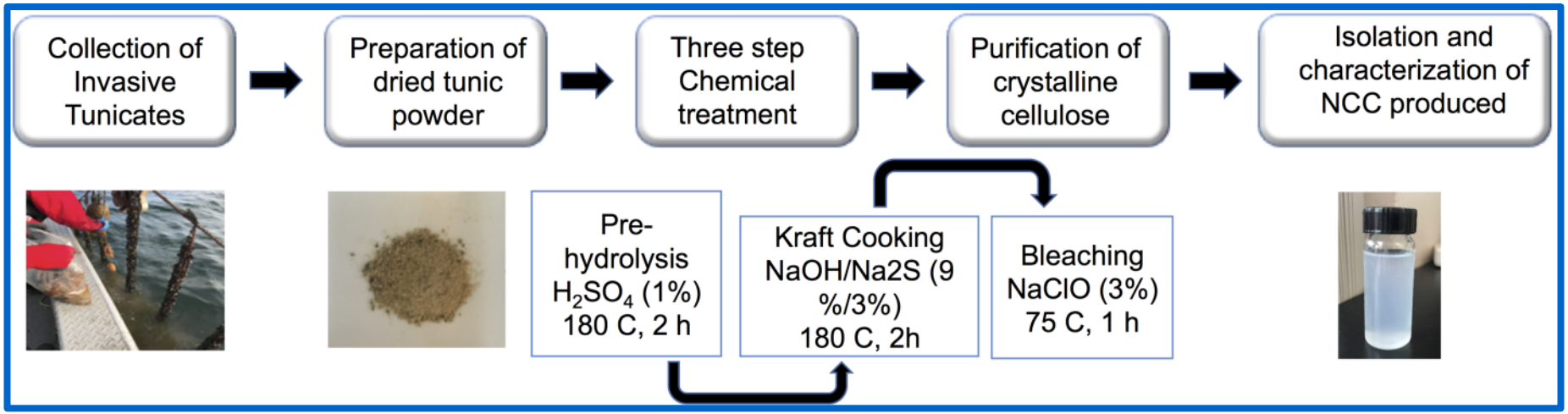
"The P.E.I. mussel farming industry has been hit hard since 1998 by four invasive species of tunicate, according to Fisheries and Oceans Canada. Efforts have focused on minimizing the spread and impact of tunicates"

Source: CBC News. Posted: Mar 22, 2014 9:55 AM AT



From Nuisance to Resource

- Tunicates constitute the only known animal source of nanocrystalline cellulose (NCC).
- This research represents the first time tunicates have been treated as a resource, rather than as a nuisance to be mitigated and contained.



Steps used to isolate NCC from tunicates

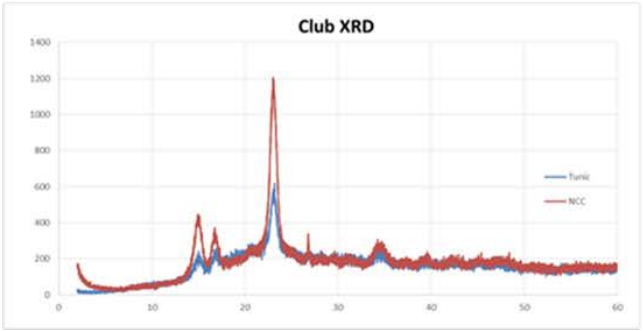
Tunicate Species	Solid yield after Hydrolysis (%)	Solid yield after Kraft Cooking (%)	Solid yield after Bleaching (%)
Vase (*Ciona intestinalis*)	67 ± 5	56 ± 4	44 ± 8
Club (*Styela clava*)	57 ± 4	42 ± 3	32 ± 7

Mass balance of the overall process

Crystallinity of Tunicate NCC

X-Ray Diffraction Analysis (XRD):

- The degree of crystallinity is 89.1% for club and 73.7% for vase
- The average crystallite size for NCC from club and vase are 112.3 Å and 94 Å



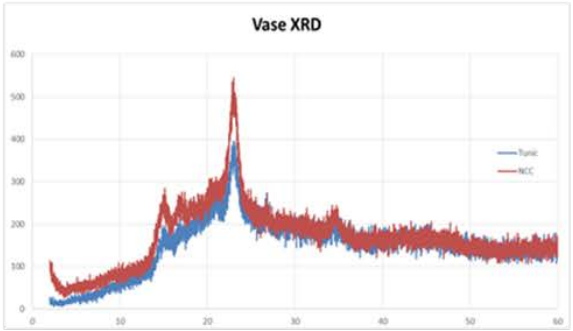


Table 7: Information values for crystalline fraction for CNCD-1

Method	Value
Solid state NMR [a]	0.59 [b]
X-ray diffraction [c]	0.88 [d]

[a] The crystalline fraction is measured by 13C cross polarization magic angle spinning solid state NMR and is the ratio of the broad signal from disordered C4 to the total C4 signal (disordered + crystalline); note that surface glucose units are included in the disordered fraction [3].

[b] A standard deviation s = 0.01 was obtained for 5 measurements, each on a different sample.

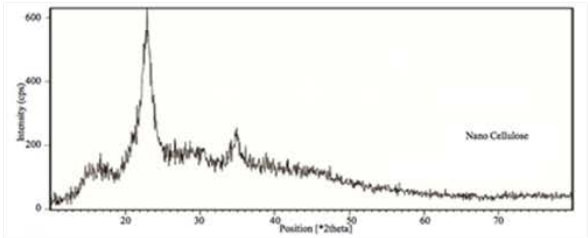
[c] A thin film sample was prepared from a CNCD-1 suspension. The crystalline fraction is obtained as the ratio of the total intensity of crystalline regions divided by the sum of crystalline and amorphous regions in the X-ray diffraction spectrum, with deconvolution using Ruland-Rietveld analysis [4].

[d] A standard deviation s = 0.02 was obtained for 5 measurements, each on a different subsample.

NCC from tunicates has surpassed the certification values.

CNCD-1: Cellulose Nanocrystal Powder Certified Reference Material
https://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/cncd_1.html

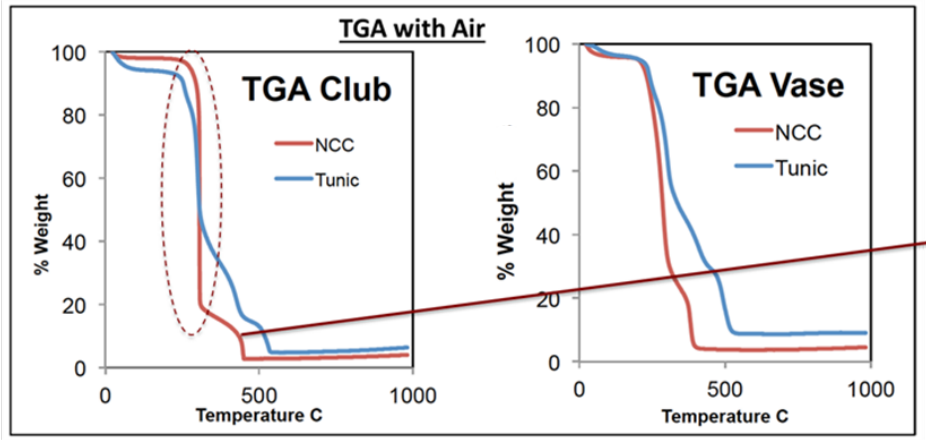
NCC from tunicates has higher degree of crystallinity than obtained from paper

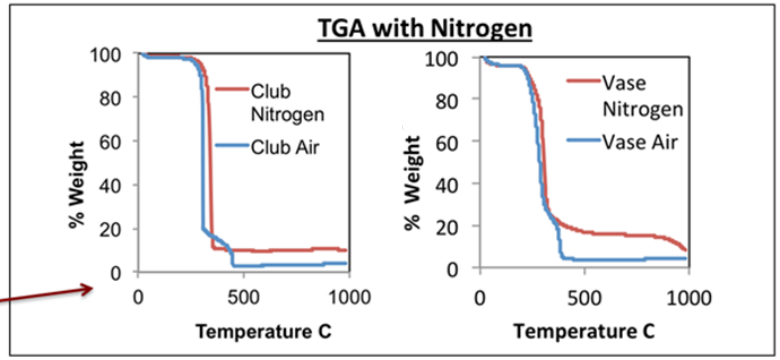


The crystallinity of NCC was about 81.5%.
[Kermani & Esfandiary, 2016]

Thermal Behavior

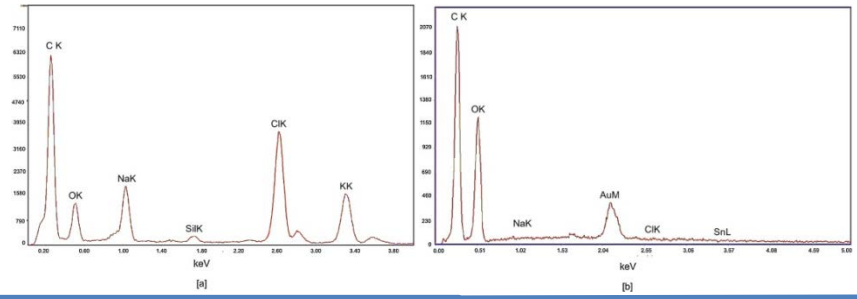
Thermogravimetric Analysis (TGA): Club NCC has higher thermal stability than NCC from Vase Tunicates





88% cellulose with 9% residual in NCC from club

EDX Analysis



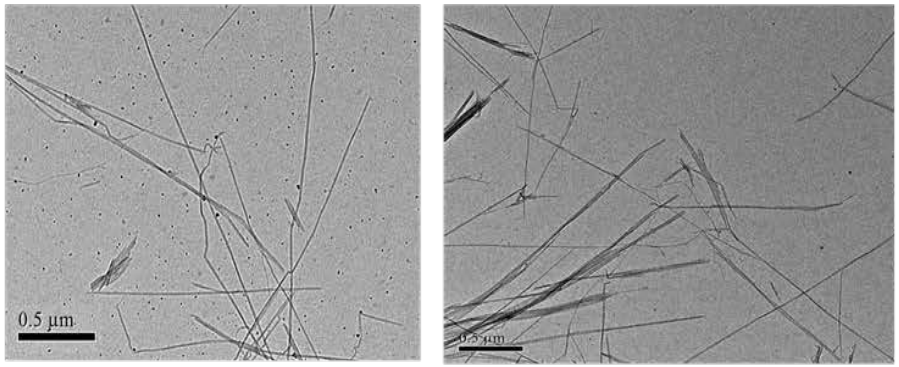
- NCC obtained from both club and vase shows weight loss of more than 80% in the temperature range of 300±25C.
- Sudden change in mass loss observed between 300 -450 C.

Surface Morphology

Transmission Electron Microscopy (TEM):

- TEM results showed Club sourced NCC displays a higher average length (1567 ± 638 nm) than vase sourced NCC (1374 ± 600 nm).
- The average width of NCC obtained was (18.3 ± 10.1 nm) and (17.4 ± 10.7 nm) for club and vase respectively.
- This results in an aspect ratio of (90 ± 57) for club and (80 ± 60) for vase tunicates.

TEM images of NCC from [a] club and [b] vase tunicates



[a] [b]

Exceeds NRC Certified Reference Material Standards

Table 5: Reference values for particle length and height of CNC-1

Parameter	Value, nm
Length AFM	133 ± 100 a c
Length TEM	108 ± 34 a d
Height AFM	4.1 ± 3.2 b c

a Length is the longest dimension of a particle deposited on a substrate measured along a direction parallel to the substrate. Value presented is the arithmetic mean and uncertainty corresponds to the standard deviation.

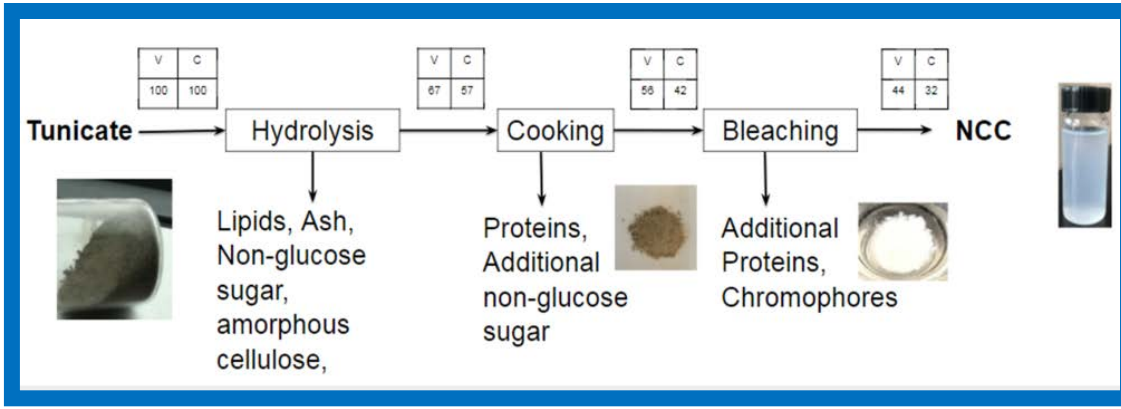
b Height is the arithmetic mean of a short dimension of a particle deposited on a substrate measured along a direction perpendicular to the length and parallel to the substrate normal. Uncertainty corresponds to the standard deviation of analysis of 32 individual particles.

c Value derived from image analysis of 32 individual particles.

d Value derived from image analysis of 1672 individual particles.

Comparison to Literature

Tunicate source	NCC yield	Isolation Method	Average length of NCC	Reference
Styela clava (Club)	32 ± 7%	H_2SO_4 prehydrolysis-kraft cooking-bleaching	1567 ± 638 nm	This work
Ciona intestinalis (Vase)	44 ± 8%	H_2SO_4 prehydrolysis-kraft cooking-bleaching	1374 ± 600 nm	This work
Microscosmus fulcatus	Not provided	H_2SO_4 hydrolysis	1160nm	[1]
Styela plicata	24%	H_2SO_4 prehydrolysis-kraft cooking-bleaching	1000-3000nm	[2]
Ciona intestinalis (Vase)	12%	H_2SO_4 prehydrolysis-kraft cooking-bleaching	>1000nm	[2]
Halocynthia roretzi	21%	H_2SO_4 prehydrolysis-kraft cooking-bleaching	1073nm	[3]

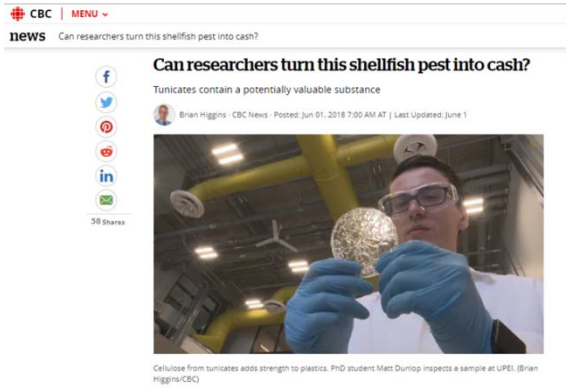


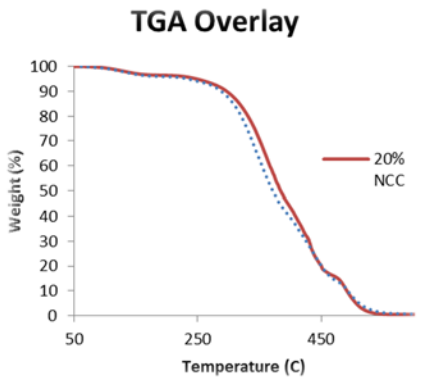
Yields of Club (C) and Vase (V) tunicate derived NCC and intermediaries.

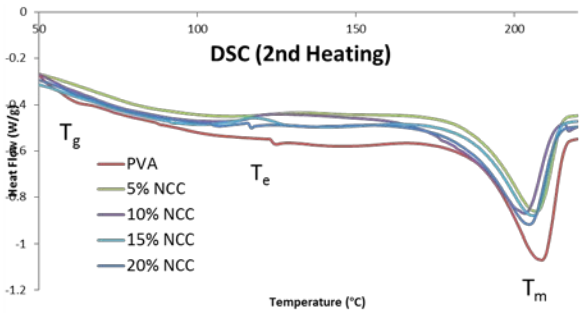
[1] M. M. D. Lima et al. *Langmuir*. 19 (2003) 24–29.
[2] Y. Zhao and J. Li. *Cellulose*. 21 (2014) 3427–3441.
[3] S. Elazzouzi-Hafraoui et al. *Biomacromolecules*. 9 (2008) 57-65.

Applications of Tunicate Sourced NCC

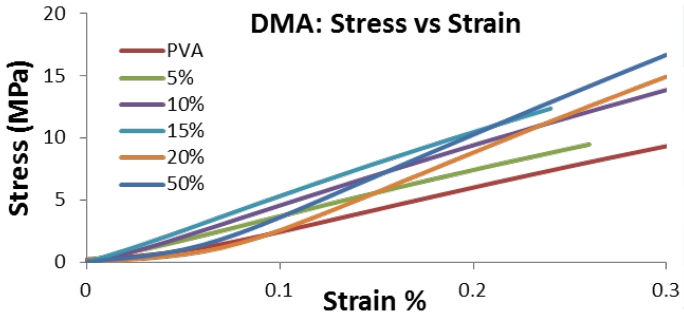
A high strength UV-absorbing material comprised of tunicate sourced NCC and polyvinyl alcohol is under development for use in durable UV absorbent coating applications.



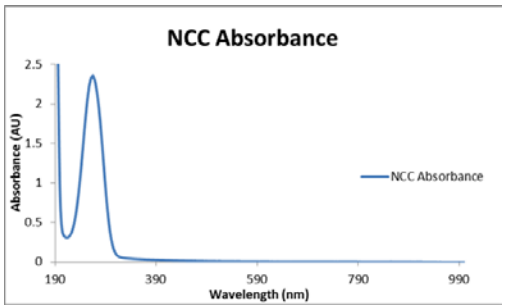








Sample	Youngs Modulus (Gpa) [0.05-0.3% Strain]	Standard Deviation
PVA	3.45	0.30
5% NCC	3.97	0.26
10% NCC	4.71	0.32
15% NCC	5.04	0.36
20% NCC	5.55	0.57
50% NCC	5.93	0.63



Additional Applications of Tunicate-NCC

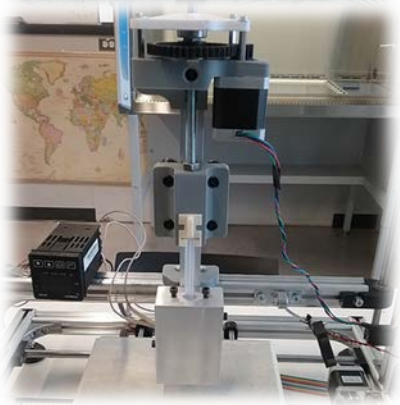
Bioprinting Applications (**Ali Ahmadi**)

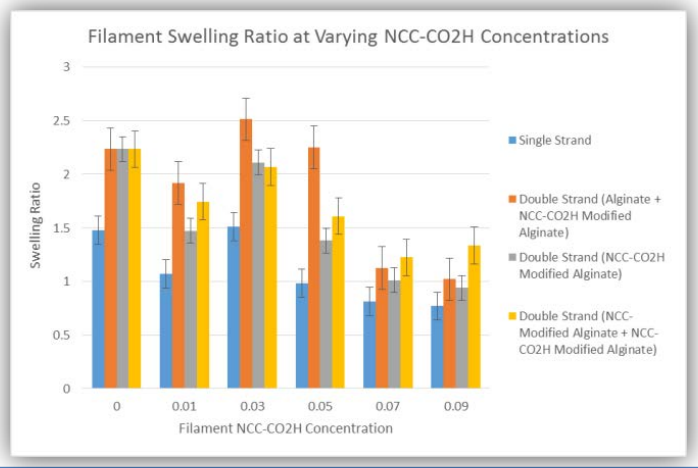
Humidity and pH responsive bioinks derived from club tunicate sourced NCC and sodium alginate have been produced for use in 3D and 4D bioprinting applications.

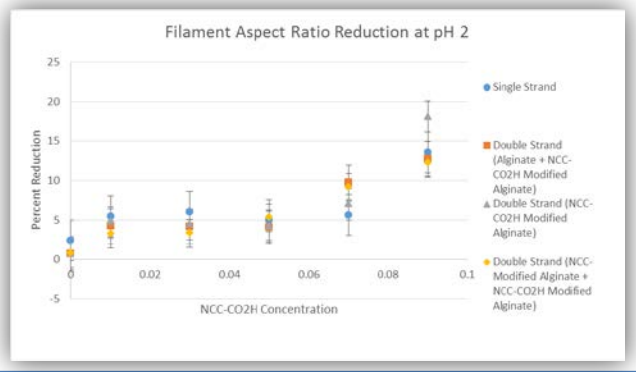
This work is being carried out by Dr. Ali Ahmadi, at UPEI.











Additional Applications of Tunicate-NCC

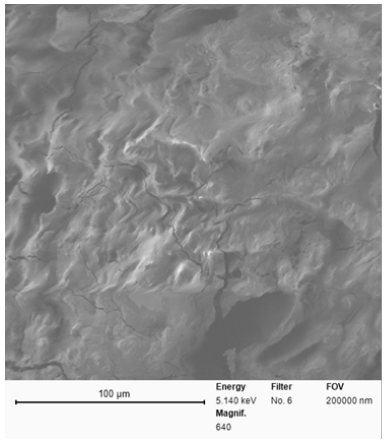
Biomedical Applications (**Marya Ahmed**)

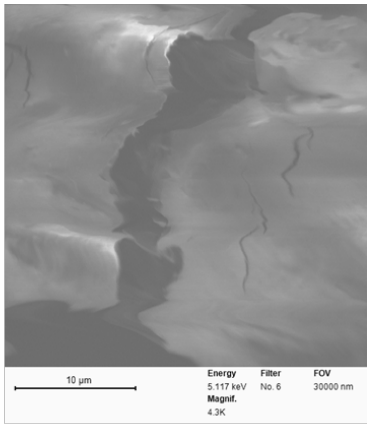
A biomedical scaffold for use in skin tissue engineering applications has been produced from tunicate sourced NCC, dopamine and NH_4OH. Work is currently ongoing, and in early stages of development.

This work is being carried out by Dr. Marya Ahmed, at UPEI.









M. Ahmed Laboratory

University of Prince Edward Island

	DOPA [µM]	TEPMA:MA [uM]	NCC (w/v%)	KPS (mol %)	TEMEDA	NH_4OH [mM]	*N,N'*-Methylenebisacrylamide (mol %)	Gelation Time (minutes)
A'	68	90:0)	10	10	20 µl	1.14	10	NA
B'	68	90: 45	10	10	20 µl	1.14	10	NA
C'	68	90 90	10	10	20 µl	1.14	10	NA
D'	68	90 180	10	10	20 µl	1.14	10	45
E'	68	90 360	10	10	20 µl	1.14	10	45

Future Outlook

Invasive tunicates on PEI are not going away.

Island aquaculture and our local economy continue to face growing challenges.

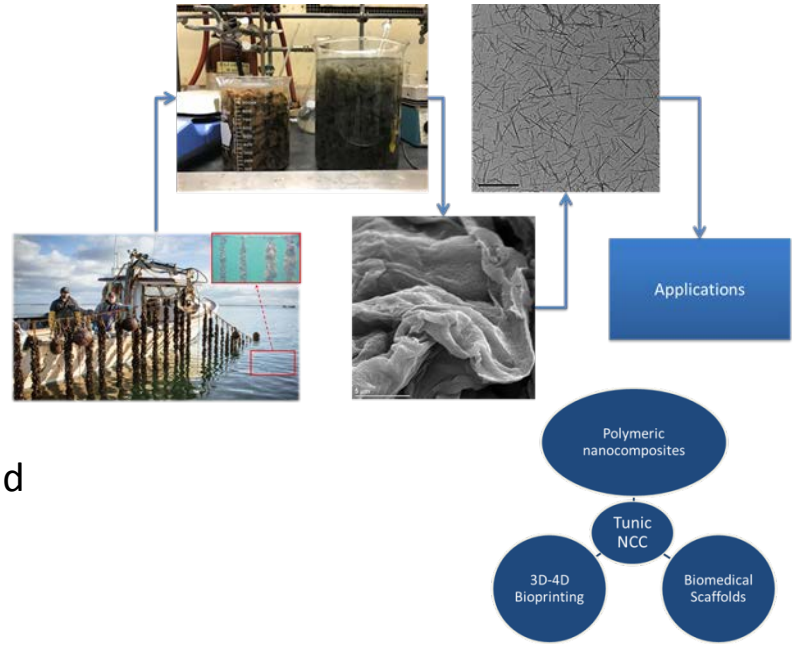
Value added products including NCC can be isolated from invasive tunicates.

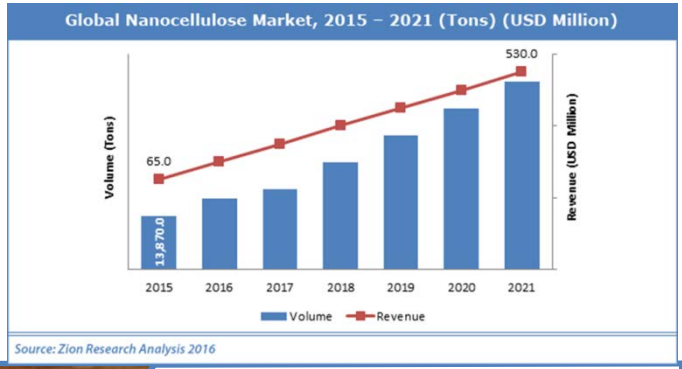
Optimization and scale up of our tunicate to NCC process; could directly help, and eventually solve the tunicate problem.

Creating new areas of economic and research potential.

While providing a superior NCC product to growing global markets.

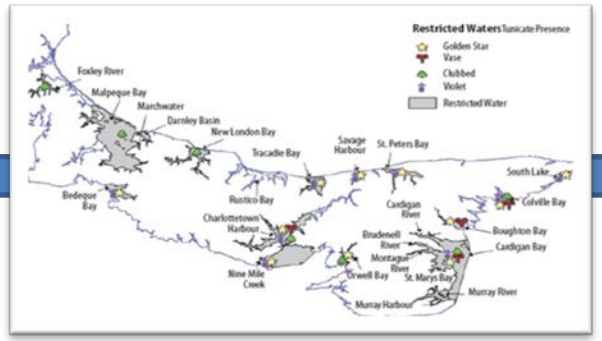
Driving the *Circular Economy for Bioproducts Innovation*.



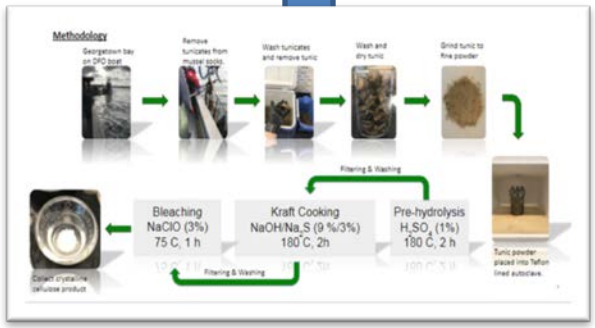


Thank you









Acknowledgements

Supervisory Committee

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- *Dr. Amy Hsiao*
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- Dr. Gobinda Saha
- Mr. Steven Cogswell
- Mr. Stephen Scully
- Mr. Chuck Terrio
- Mr. Peter Warris
- Wyatt Mcnevin

- PEI Aquaculture Alliance
- Department of Fisheries and Oceans
- UPEI Faculty of Sustainable Design Engineering
- UPEI Department of Chemistry
- UNB Microscopy and Microanalysis Facility

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